Data Evaluation Report on the Acute Toxicity of Ethylenethiourea to Aquatic Vascular Plants (*Lemna gibba*)

PMRA Submission Number N/A EPA MRID Number 47441203

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D353731
	OECD Data Point	{.....}
	EPA MRID	47441203
	EPA Guideline	OPPTS 850.4400 (123-2)

Test material: Ethylenethiourea; 2-imidazolidinethione **Purity:** 100%
Common name: Ethylenethiourea
Chemical name: IUPAC: Not Reported
CAS name: Not Reported
CAS No.: 96-45-7 (2-imidazolidinethione)
Synonyms: None Reported

Primary Reviewer: John Marton **Signature:**
Staff Scientist, Cambridge Environmental, Inc. **Date:** 07/22/08

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental, Inc. **Date:** 03/03/09

Primary Reviewer: Brian Montague, Fishery Biologist, USEPA **Date:** February, 2015

Secondary Reviewer(s): {.....} **Date:** {.....}
{EPA } 04/16/15

Reference/Submission No.:

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	600016	

Date Evaluation Completed: 12-02- 2015

CITATION: Softcheck, K.A. 2008. Ethylenethiourea- 7-Day Toxicity Test with Duckweed (*Lemna gibba*) Following OPPTS Draft Guideline 850.4400. Unpublished study performed by Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts 02571-1037. Laboratory report number 13921.6101. Study submitted to EBDC/ETU Task Force, c/o McDermott, Will and Emery LLP, 600 13th St NW, Washington, DC 20005. Study submitted June 2, 2008.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic vascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 7- day acute toxicity study, the freshwater floating aquatic vascular plants, duckweed (*Lemna gibba*) were exposed to ethylenethiourea at nominal concentrations of 0 (negative control), 63, 130, 250, 500 and 1000 mg ai/L under static-renewal conditions; the mean-measured concentrations were <6.7 (<LOQ; control), 59, 120, 230, 480 and 960 mg ai/L. The NOAEC and EC_{50} values based on frond density (the most sensitive endpoint) were 230 and >960 mg ai/L, respectively. The % growth inhibition, based on frond density, in the treated culture as compared to the control ranged from -14 to 28%.

After 7 days of exposure, mean frond density was 390 fronds/rep in the negative control and 445, 441, 434, 349 and 280 fronds/rep in the mean measured 59, 120, 230, 480 and 960 mg ai/L treatment groups, respectively, yielding inhibitions of -14, -13, -11, 11 and 28%, respectively. The Day 0-7 growth rate averaged 0.47 in the negative control and 0.49, 0.48, 0.48, 0.45 and 0.42 in the mean measured 59, 120, 230, 480 and 960 mg ai/L treatment groups, respectively, yielding inhibitions of -4, -2, -2, 4 and 11%, respectively. The Day 7 dry weight averaged 0.0372 g in the negative control and 0.0497, 0.0460, 0.0452, 0.0396 and 0.0352 g in the mean measured 59, 120, 230, 480 and 960 mg ai/L treatment groups, respectively, yielding inhibitions of -34, -24, -22, -7 and 5%, respectively. On Days 3, 5 and 7, curled fronds were observed in the mean-measured 960 mg ai/L treatment group. No other phytotoxic effects were reported.

This toxicity study is classified as scientifically sound and satisfies the guideline requirement for an acute aquatic vascular plant toxicity study.

Results Synopsis

Test Organism: *Lemna gibba*
Test Type (Flow-through, Static, Static Renewal): Static Renewal

Frond Density (reviewer-reported):
EC_{05}: 260 mg ai/L 95% C.I.: 120-570 mg ai/L
EC_{50}: >960 mg ai/L 95% C.I.: N/A
NOAEC: 230 mg ai/L
LOAEC: 480 mg ai/L
Probit Slope: 2.29±0.640

Growth Rate (reviewer-reported):
EC_{05}: 480 mg ai/L 95% C.I.: 300-770 mg ai/L
EC_{50}: >960 mg ai/L 95% C.I.: N/A
NOAEC: 230 mg ai/L
LOAEC: 480 mg ai/L
Probit Slope: 1.84±0.541

Dry Weight (study author-reported):
EC_{05}: 340 mg ai/L 95% C.I.: 250-320 mg ai/L
EC_{50}: >960 mg ai/L 95% C.I.: N/A
NOAEC: 960 mg ai/L
LOAEC: >960 mg ai/L
Probit Slope: Not Reported

Endpoint(s) Effected: Frond Density and Growth Rate

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in U.S. EPA OPPTS Draft Guideline 850.4400. The following deviations from OPPTS 850.4400 were noted:

1. The pre-test health of the duckweed culture was not specified.
2. The results from a periodic screening analysis of the dilution water were not provided.
3. It was not reported if test vessels were aerated and/or agitated.
4. The actual number of plants per replicate was not specified. Rather, the study author reported that plants with 3 to 4 fronds were introduced until each replicate contained 15 fronds.

These deviations do/do not impact the acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with all pertinent U.S. EPA Good Laboratory Practice regulations (40 CFR, Part 160) with the following exceptions: routine dilution water contaminant screening analyses for pesticides, PCBs and toxic metals were conducted at GeoLabs, Inc., Braintree, Massachusetts using standard U.S. EPA Procedures and are considered facility records. Since the analyses were conducted following standard validated methods, these exceptions had no impact on the study results

A. MATERIALS:

1. Test material Ethylenethiourea

Description: Solid

Lot No./Batch No. : 04816CH

Purity: 100%

Stability of compound under test conditions: Stable. Method validation was conducted by determining the recovery of ethylenethiourea from 0.1% trifluoroacetic acid in 20 ppt filtered seawater at fortified concentrations of 0, 0.300, 20.0 and 1000 mg/L. Individual recoveries ranged from 74.7 to 103% of nominal with a mean recovery of 93.4%

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Stored at room temperature in the dark.

Physicochemical properties of Ethylenethiourea.

Parameter	Values	Comments
Water solubility at 20°C	Not Reported	

Parameter	Values	Comments
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

2. Test organism:

Name: Duckweed (*Lemna gibba*) *EPA requires a vascular species: Lemna gibba.*
Strain, if provided: Strain 310
Source: Fronds were maintained in stock culture at Springborn Smiths, but were originally obtained from the University of Toronto, Toronto, Canada
Age of inoculum: 6 Days
Method of cultivation: 20X Algal Assay Procedure (AAP) Medium

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 7-day range-finding test was conducted with nominal concentrations of 0 (negative control), 0.010, 0.10, 1.0, 10 and 100 mg ai/L under static renewal conditions (test solutions were renewed on Days 3 and 5). After 7 days of exposure, frond density was 344, 340, 331, 362 and 341 fronds/rep in the 0.010, 0.10, 1.0, 10 and 100 mg ai/L treatment groups, respectively, compared to 333 fronds/rep in the negative control. Fronds exposed to the 100 mg ai/L treatment level were observed to be slightly chlorotic, while fronds in the control and remaining treatment levels appeared normal and healthy.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous Same as test Not Reported	
Test system		

Parameter	Details	Remarks *Criteria*
Static/static renewal Renewal rate for static renewal	Static Renewal Test solutions were renewed on Days 3 and 5.	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Temperature-controlled environmental chamber	
Duration of the test	7 Days	*EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
<u>Test vessel</u> Material: *(glass/stainless steel)* Size: Fill volume:	 Glass crystallizing dishes 270 mL 100 mL	
<u>Details of growth medium name</u> pH at test initiation: pH at test termination: Chelator used: Carbon source:	20X AAP 7.5-8.0 (new solutions on Days 0, 3 and 5) 8.3-9.2 (aged solutions on Days 3, 5 and 7) Yes $NaHCO_3$	*EPA recommends the following culture media: Modified Hoagland's E+ or 20X-AAP. Chelating agents (e.g. EDTA) are recommended in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Standard nutrient medium was used and a detailed composition was provided.	
<u>Dilution water</u>	Ingredients to prepared 20X AAP	

Parameter	Details	Remarks *Criteria*
source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	medium were added to sterile, deionized water pH was adjusted to 7.5±0.1 at test initiation if necessary None Reported 2.5 and 2.7 mg/L in February and March 2008, respectively Not Reported Not Reported Not Reported Not Reported	*EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Appropriate amounts of a stock solution (1000 mg ai/L) were diluted with 20X AAP medium to achieve the desired nominal concentrations.	
Aeration or agitation	Not specified	
Sediment used (**for rooted aquatic vascular plants**)	N/A	
Number of replicates Control: Solvent control: Treatments:	 3 N/A 3	
Number of plants/replicate	Not specified – probably 4-5 plants based on total fronds below	*EPA requires 5 plants.*
Number of fronds/plant	An inoculum of plants with three to four fronds each was introduced into the test vessels until each vessel contained 15 fronds.	*EPA requires 3 fronds per plant.*
Test concentrations Nominal:	0 (negative control), 63, 130, 250, 500 and 1000 mg ai/L	

Parameter	Details	Remarks *Criteria*
Measured:	<6.7 (<LOQ; control), 59, 120, 230, 480 and 960 mg ai/L	*EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	N/A; a solvent control was not used	
Method and interval of analytical verification	Samples were collected from new solutions at test initiation (0 hours) and samples were collected from aged solutions on Day 3. See Reviewer's Comments section for further details.	
Test conditions Temperature: Photoperiod: Light intensity and quality:	 24-25°C Continuous light 5100-6600 lux	
Reference chemical (if used) name: concentrations:	 N/A; a reference chemical was not used	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (e.g.,: number of fronds, plant dry weight or other toxicity symptoms)	-Frond Density -Average Growth Rate -Dry Weight -Phytotoxicity	
Measurement technique for frond number and other end points	Frond count and phytotoxicity were assessed by direct observation of the replicate test vessels. Dry weight was determined by drying fronds in an oven at 62 to 63°C for three days in preweighed aluminum pans and then reweighing the	

Parameters	Details	Remarks/Criteria
	pans. Growth was determined based on the frond density.	
Observation intervals	Frond counts were made on Days 3, 5 and 7; assessments of phytotoxicity were made along with frond counts. Dry weight and growth rate were determined at test termination.	
Other observations, if any	None reported	
Indicate whether there was an exponential growth in the control	Mean frond density in the negative increased from 15 fronds/rep at test initiation to 390 fronds/rep at test termination.	
Were raw data included?	Replicate data were provided	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

After 7 days of exposure, mean frond density was 390 fronds/rep in the negative control and 445, 441, 434, 349 and 280 fronds/rep in the mean measured 59, 120, 230, 480 and 960 mg ai/L treatment groups, respectively, yielding inhibitions of -14, -13, -11, 11 and 28%, respectively. The study author reported frond density NOAEC, EC_{05} and EC_{50} values of 960, 300 and >960 mg ai/L, respectively.

The Day 0-7 growth rate averaged 0.47 in the negative control and 0.49, 0.48, 0.48, 0.45 and 0.42 in the mean measured 59, 120, 230, 480 and 960 mg ai/L treatment groups, respectively, yielding inhibitions of -4, -2, -2, 4 and 11%, respectively. The study author reported frond density NOAEC, EC_{05} and EC_{50} values of 960, 430 and >960 mg ai/L, respectively.

The Day 7 dry weight averaged 0.0372 g in the negative control and 0.0497, 0.0460, 0.0452, 0.0396 and 0.0352 g in the mean measured 59, 120, 230, 480 and 960 mg ai/L treatment groups, respectively, yielding inhibitions of -34, -24, -22, -7 and 5%, respectively. The study author reported frond density NOAEC, EC_{05} and EC_{50} values of 960, 340 and >960 mg ai/L, respectively.

On Days 3, 5 and 7, curled fronds were observed in the mean-measured 960 mg ai/L treatment group. No other phytotoxic effects were reported.

Table 3: Effect of Ethylenethiourea on frond number of duckweed (*Lemna gibba*)

Mean-Measured and (Nominal) Concentrations mg ai/L	Initial Frond Number/Test Solution	Frond Number at			
		Day 3	Day 5	Day 7	
				Frond Number	% Inhibition [a]
Negative control	15	70	180	390	N/A
59 (63)	15	64	181	445	-14
120 (130)	15	70	187	441	-13
230 (250)	15	64	176	434	-11
480 (500)	15	56	143	349	11
960 (1000)	15	52	127	280	28
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

[a] Negative percent inhibition indicates promoted growth
N/A- Not Applicable

Table 4: Effect of Ethylenethiourea on growth of duckweed (*Lemna gibba*)

Mean-Measured and (Nominal) Concentrations mg ai/L	Initial Frond Number/Test Solution	Mean Growth Rate ($days^{-1}$)		Frond Dry Weight (g)	
		Days 0-7	% Inhibition [a]	Day 7	% Inhibition [a]
Negative control	15	0.47	N/A	0.0372	N/A
59 (63)	15	0.49	-4	0.0497	-34
120 (130)	15	0.48	-2	0.0460	-24
230 (250)	15	0.48	-2	0.0452	-22
480 (500)	15	0.45	4	0.0396	-7
960 (1000)	15	0.42	11	0.0352	5

N/A- Not Applicable
[a] Negative percent inhibition indicates promoted growth

Table 5: Statistical endpoint values.

Statistical Endpoint	Frond Density	Growth Rate	Dry Weight
NOAEC / EC_{05} (95% C.I.) (mg ai/L)	960 / 300 (250-350)	960 / 430 (370-520)	960 / 340 (160-570)
LOAEC (mg ai/L)	>960	>960	>960
IC_{50} or EC_{50} (mg ai/L) (95% C.I.)	>960	>960	>960
Other (IC_{90}/EC_{90})	>960	>960	>960
Reference chemical NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

N/A- Not Applicable

B. REPORTED STATISTICS:

Means and standard deviations were calculated for frond density and growth rate at each observation interval and for dry weight at test termination. Data for these three endpoints were checked for normality using Shaprio-Wilks' Test (Weber, et al., 1989) and for homogeneity of variance using Bartlett's test (Horning and Weber, 1985). If the data sets passed the tests for homogeneity and normality, then Williams' Test was used to determine the NOAEC and LOAEC values. If the data did not pass the tests for homogeneity and normality, then Kruskal-Wallis' Test was used to determine the NOAEC. All statistical determinations were made at the 95% level of certainty, except in the case of the Shapiro-Wilks' and Bartlett's Tests, where the 99% level of certainty was applied. TOXSTAT® version 3.5 (Gulley et al. 1996) was used to perform both the statistical (LOAEC and NOAEC determinations) and ECx calculations. If no concentration resulted in a 5%, 50% or 90% reduction, the EC values were empirically estimated to be greater than the highest concentration tested.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The reviewer tested the frond density, growth rate and dry weight data sets for normality using the Chi-Square and Shapiro-Wilks tests and for homogeneity of variance using the Hartley and Bartlett's tests. If the data met these assumptions of ANOVA, the NOAEC value was determined using the parametric Dunnett's and Williams' tests. If the data did not meet these assumptions, the NOAEC value was determined using the non-parametric Kruskal-Wallis test. NOAEC determinations were made using Toxstat statistical software. The reviewer then attempted to determine ECx values and probit slopes using the probit analysis via Nuthatch statistical software. All analyses were conducted using the mean-measured concentrations.

Frond Density:
EC_{05}: 260 mg ai/L 95% C.I.: 120-570 mg ai/L
EC_{50}: >960 mg ai/L 95% C.I.: N/A
NOAEC: 230 mg ai/L
LOAEC: 480 mg ai/L
Probit Slope: 2.29±0.640

Growth Rate:
EC_{05}: 480 mg ai/L 95% C.I.: 300-770 mg ai/L
EC_{50}: >960 mg ai/L 95% C.I.: N/A
NOAEC: 230 mg ai/L
LOAEC: 480 mg ai/L
Probit Slope: 1.84±0.541

Dry Weight:
EC_{05}: 380 mg ai/L 95% C.I.: 64-2200 mg ai/L
EC_{50}: >960 mg ai/L 95% C.I.: N/A
NOAEC: 960 mg ai/L
LOAEC: >960 mg ai/L
Probit Slope: 2.11±1.83

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's NOAEC and LOAEC values for frond density and growth rate were more conservative than those of the study author; therefore, the reviewer's results for these two endpoints are reported in the Executive Summary and Conclusions sections of this DER. The reviewer's NOAEC and LOAEC values for dry weight were identical to those of the study author; however, the study author reported a narrower 95% confidence interval associated with the EC_{05}. Therefore the study author's results fro dry weight are reported in the Executive Summary and Conclusions sections of this DER.

The reviewer's non-parametric analysis (Kruskal-Wallis Test) of growth rate did not detect any significant inhibitions at any treatment level relative to the negative control. However, the EC_{10} and EC_{25} values were 380 and 700 mg ai/L, respectively, which the reviewer felt were biologically significant inhibition concentrations. Therefore, the highest mean-measured concentration below the EC_{10} value (230 mg ai/L) was visually determined by the reviewer as the NOAEC value.

Test solutions were renewed on Days 3 and 5, but analytical verification was not conducted before and after each

renewal. Instead, new solutions were analyzed 0 and 24 hours and aged solutions were analyzed on Day 3. The measured concentrations at the nominal 500 and 1000 mg ai/L treatment levels were 950 and 490 mg ai/L, respectively, at 0 hours. These two levels were re-sampled on Day 1 and measured concentrations were 480 and 940 mg ai/L, respectively, suggesting that the Day 0 samples were inadvertently mishandled. Therefore, the 0 hour values for the two highest levels were switched to represent the correct measured concentrations and these 0 hour values were used to estimate the mean-measured concentrations. The mean-measured concentrations ranged from 92 to 96% of nominal for Days 0-3. Because this was the longest interval between any renewal period (3 days), the reviewer feels that the use of the mean-measured concentrations based on the samples from test initiation and Day 3 is acceptable.

The in-life portion of the definitive toxicity test was conducted from February 26 to March 7, 2008 (including dry weight determination).

F. CONCLUSIONS:

The study is scientifically sound and acceptable for use in risk assessment. The NOAEC and EC_{50} values based on frond density (the most sensitive endpoint) were 230 and >960 mg ai/L, respectively.

Frond Density (reviewer-reported):
EC_{05}: 260 mg ai/L 95% C.I.: 120-570 mg ai/L
EC_{50}: >960 mg ai/L 95% C.I.: N/A
NOAEC: 230 mg ai/L
LOAEC: 480 mg ai/L
Probit Slope: 2.29±0.640

Growth Rate (reviewer-reported):
EC_{05}: 480 mg ai/L 95% C.I.: 300-770 mg ai/L
EC_{50}: >960 mg ai/L 95% C.I.: N/A
NOAEC: 230 mg ai/L
LOAEC: 480 mg ai/L
Probit Slope: 1.84±0.541

Dry Weight (study author-reported):
EC_{05}: 340 mg ai/L 95% C.I.: 250-320 mg ai/L
EC_{50}: >960 mg ai/L 95% C.I.: N/A
NOAEC: 960 mg ai/L
LOAEC: >960 mg ai/L
Probit Slope: Not Reported

Endpoint(s) Effected: Frond Density and Growth Rate

III. REFERENCES:

ASTM. 2002. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19426.

Gulley, D.D., Boelter, A.M. and Bergman, H.L. 1996 TOXSTAT® Release 3.5. University of Wyoming, Laramie, Wyoming.

Horning, W.B. and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-85/014. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

U.S. EPA. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Guideline, OPPTS 850.4400. Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II. "Public Draft". EPA 712-C-96-118. April 1996. U.S. Environmental Protection Agency, Washington, DC.

Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning II, F.A. Kessler, J.R. Menkedick, T.W. Heiheisel, P.A. Lewis, D.J. Klemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer and R.W. Freyberg (eds.). 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2nd ed. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
Frond density (fronds/rep), Day 7; mg ai/L
File: 1203fc       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
--------------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5    >0.5 to 1.5    >1.5
             _____     _____________     ___________    ___________    _____

EXPECTED     1.206         4.356             6.876          4.356      1.206
OBSERVED     0             8                 4              6          0

--------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   7.2838
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.



Frond density (fronds/rep), Day 7; mg ai/L
File: 1203fc       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D = 6816.000

W =    0.812

Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858
--------------------------------------------------------------------------------

Data FAIL normality test. Try another transformation.

Warning -  The two homogeneity tests are sensitive to non-normal data and
           should not be performed.



Frond density (fronds/rep), Day 7; mg ai/L
File: 1203fc        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =    2.26
Closest, conservative, Table H statistic = 1362.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   6,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   6,    df (# avg reps-1) =   2.00

--------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).
```

Frond density (fronds/rep), Day 7; mg ai/L
File: 1203fc Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 0.51
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
used to calculate the B statistic (see above).

Frond density (fronds/rep), Day 7; mg ai/L
File: 1203fc Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	64221.111	12844.222	22.613
Within (Error)	12	6816.000	568.000	
Total	17	71037.111		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

Frond density (fronds/rep), Day 7; mg ai/L
File: 1203fc Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	389.667	389.667		
2	59	445.333	445.333	-2.861	
3	120	441.000	441.000	-2.638	
4	230	434.000	434.000	-2.278	
5	480	348.667	348.667	2.107	
6	960	280.000	280.000	5.636	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Frond density (fronds/rep), Day 7; mg ai/L

File: 1203fc Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	3			
2	59	3	48.648	12.5	-55.667
3	120	3	48.648	12.5	-51.333
4	230	3	48.648	12.5	-44.333
5	480	3	48.648	12.5	41.000
6	960	3	48.648	12.5	109.667

Frond density (fronds/rep), Day 7; mg ai/L
File: 1203fc Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	389.667	389.667	427.500
2	59	3	445.333	445.333	427.500
3	120	3	441.000	441.000	427.500
4	230	3	434.000	434.000	427.500
5	480	3	348.667	348.667	348.667
6	960	3	280.000	280.000	280.000

Frond density (fronds/rep), Day 7; mg ai/L
File: 1203fc Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	427.500				
59	427.500	1.944	*	1.78	k= 1, v=12
120	427.500	1.944	*	1.87	k= 2, v=12
230	427.500	1.944	*	1.90	k= 3, v=12
480	348.667	2.107	*	1.92	k= 4, v=12
960	280.000	5.636	*	1.93	k= 5, v=12

s = 23.833
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	2.6E+02	1.2E+02	5.7E+02	0.16	0.47
EC10	3.8E+02	2.2E+02	6.7E+02	0.11	0.57
EC25	7.0E+02	5.4E+02	9.1E+02	0.054	0.77
EC50	1.4E+03	9.9E+02	1.9E+03	0.067	0.72

Slope = 2.29 Std.Err. = 0.640

!!!Poor fit: p = 0.022 based on DF= 3.0 12.

1203FC : Frond density (fronds/rep), Day 7; mg ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	390.	429.	-39.4	100.	0.00
59.0	3.00	445.	429.	16.6	99.9	0.0853
120.	3.00	441.	426.	15.1	99.2	0.752
230.	3.00	434.	413.	20.9	96.3	3.72
480.	3.00	349.	366.	-17.7	85.4	14.6
960.	3.00	280.	275.	4.52	64.2	35.8

!!!Warning: EC50 not bracketed by doses evaluated.

Growth rate (days^-1), Days 0-7; mg ai/L
File: 1203gr Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

---

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.206	4.356	6.876	4.356	1.206
OBSERVED	0	11	1	6	0

Calculated Chi-Square goodness of fit test statistic = 18.1877
Table Chi-Square value (alpha = 0.01) = 13.277

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

Growth rate (days^-1), Days 0-7; mg ai/L
File: 1203gr Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.001

W = 0.772

Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

Growth rate (days^-1), Days 0-7; mg ai/L
File: 1203gr Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 4.00
Closest, conservative, Table H statistic = 1362.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 2
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

Growth rate (days^-1), Days 0-7; mg ai/L
File: 1203gr Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 0.97
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
used to calculate the B statistic (see above).

Growth rate (days^-1), Days 0-7; mg ai/L
File: 1203gr Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	0.467	0.467	26.000
2	59	0.487	0.487	41.500
3	120	0.487	0.487	41.500
4	230	0.483	0.483	40.000
5	480	0.450	0.450	16.000
6	960	0.417	0.417	6.000

Calculated H Value = 14.232 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

Growth rate (days^-1), Days 0-7; mg ai/L
File: 1203gr Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 6 5 1 4 3 2
6	960	0.417	0.417	\
5	480	0.450	0.450	. \
1	neg control	0.467	0.467	. . \
4	230	0.483	0.483	. . . \
3	120	0.487	0.487	. . . . \
2	59	0.487	0.487	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 4.217

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	4.8E+02	3.0E+02	7.7E+02	0.097	0.62
EC10	7.5E+02	5.9E+02	9.6E+02	0.050	0.78
EC25	1.6E+03	1.1E+03	2.4E+03	0.079	0.68
EC50	3.8E+03	1.5E+03	9.1E+03	0.18	0.41

Slope = 1.84 Std.Err. = 0.541

!!!Poor fit: p = 0.046 based on DF= 3.0 12.

1203GR : Growth rate (days^-1), Days 0-7; mg ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	0.467	0.481	-0.0148	100.	0.00
59.0	3.00	0.487	0.481	0.00545	100.	0.0463
120.	3.00	0.487	0.480	0.00667	99.7	0.301
230.	3.00	0.483	0.475	0.00813	98.7	1.30
480.	3.00	0.450	0.457	-0.00719	95.0	5.04
960.	3.00	0.417	0.415	0.00172	86.2	13.8

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

Frond dry weight (mg), Day 7; mg ai/L
File: 1203dw Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5

EXPECTED	1.206	4.356	6.876	4.356	1.206
OBSERVED	0	6	6	6	0

Calculated Chi-Square goodness of fit test statistic = 3.7645
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Frond dry weight (mg), Day 7; mg ai/L
File: 1203dw Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 244.893

W = 0.972

Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858

Data PASS normality test at P=0.01 level. Continue analysis.

Frond dry weight (mg), Day 7; mg ai/L
File: 1203dw Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 28.96
Closest, conservative, Table H statistic = 1362.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 2
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

Frond dry weight (mg), Day 7; mg ai/L
File: 1203dw Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 4.37
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Frond dry weight (mg), Day 7; mg ai/L
File: 1203dw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	480.767	96.153	4.712
Within (Error)	12	244.893	20.408	
Total	17	725.660		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

Frond dry weight (mg), Day 7; mg ai/L
File: 1203dw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	37.200	37.200		
2	59	49.733	49.733	-3.398	
3	120	45.967	45.967	-2.377	
4	230	45.233	45.233	-2.178	
5	480	39.633	39.633	-0.660	
6	960	35.233	35.233	0.533	

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Frond dry weight (mg), Day 7; mg ai/L
File: 1203dw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	3			
2	59	3	9.221	24.8	-12.533
3	120	3	9.221	24.8	-8.767
4	230	3	9.221	24.8	-8.033
5	480	3	9.221	24.8	-2.433

```
  6                    960     3             9.221       24.8        1.967
-------------------------------------------------------------------------------


Frond dry weight (mg), Day 7; mg ai/L
File: 1203dw        Transform: NO TRANSFORMATION

        WILLIAMS TEST  (Isotonic regression model)    TABLE 1 OF 2
-------------------------------------------------------------------------------
GROUP                                ORIGINAL      TRANSFORMED    ISOTONIZED
          IDENTIFICATION       N      MEAN           MEAN           MEAN
------  --------------------  ---  -----------    -----------    -----------
   1             neg control   3      37.200         37.200         44.533
   2                      59   3      49.733         49.733         44.533
   3                     120   3      45.967         45.967         44.533
   4                     230   3      45.233         45.233         44.533
   5                     480   3      39.633         39.633         39.633
   6                     960   3      35.233         35.233         35.233
-------------------------------------------------------------------------------


Frond dry weight (mg), Day 7; mg ai/L
File: 1203dw        Transform: NO TRANSFORMATION

        WILLIAMS TEST  (Isotonic regression model)    TABLE 2 OF 2
-------------------------------------------------------------------------------
                      ISOTONIZED   CALC.     SIG      TABLE      DEGREES OF
   IDENTIFICATION       MEAN      WILLIAMS   P=.05   WILLIAMS     FREEDOM
-------------------- ----------- ----------- -----  ----------- -------------
         neg control     44.533
                  59     44.533     1.988      *       1.78       k= 1, v=12
                 120     44.533     1.988      *       1.87       k= 2, v=12
                 230     44.533     1.988      *       1.90       k= 3, v=12
                 480     39.633     0.660              1.92       k= 4, v=12
                 960     35.233     0.533              1.93       k= 5, v=12
-------------------------------------------------------------------------------
s =    4.517
Note: df used for table values are approximate when v > 20.

Estimates of EC%
-------------------------------------------------------------------------
Parameter   Estimate     95% Bounds        Std.Err.  Lower Bound
                        Lower     Upper               /Estimate
EC5          3.8E+02       64.   2.2E+03      0.36      0.17
EC10         5.6E+02   1.8E+02   1.7E+03      0.23      0.33
EC25         1.1E+03   5.7E+02   2.1E+03      0.13      0.53
EC50         2.3E+03   4.0E+02   1.3E+04      0.35      0.18

         Slope =     2.11  Std.Err. =     1.83


!!!Poor fit: p =     0.029  based on DF=       3.0       12.
-------------------------------------------------------------------------
1203DW : Frond dry weight (mg), Day 7; mg ai/L
-------------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
-------------------------------------------------------------------------
     Dose     #Reps.     Obs.      Pred.      Obs.      Pred.   %Change
                         Mean      Mean     -Pred.   %Control
```

0.00	3.00	37.2	44.5	-7.31	100.	0.00
59.0	3.00	49.7	44.5	5.24	100.	0.0412
120.	3.00	46.0	44.4	1.61	99.6	0.354
230.	3.00	45.2	43.7	1.53	98.2	1.80
480.	3.00	39.6	41.1	-1.42	92.2	7.76
960.	3.00	35.2	34.9	0.347	78.4	21.6

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.